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PATHOLOGY AND BACTERIOLOGY OF PLAGUE IN SQUIRRELS.*

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THIS report is based upon 70 plague-infected ground squirrels (*Citellus beecheyi*) which have come under observation in California during the months of June, July, and August, 1909. During this period approximately 200 squirrels have been reported as plague infected to Surgeon Rupert Blue, Public Health and Marine Hospital Service, commanding the plague suppressive measures. In the case of the majority of these, the diagnosis was based upon the gross lesions alone or upon the gross lesions in addition to the result of the microscopical examination of the smear preparations from the buboes or from other tissues. In the case of 68 of the squirrels, however, tissues were inoculated into laboratory animals and when these animals died, cultures were made from their tissues and the specific bacillus isolated in about 30 cases. In the other cases the postmortem lesions presented by the laboratory animals were so characteristic of plague that it did not seem necessary to make cultures from them. In a few cases the cultures were hopelessly contaminated and the effort to isolate the plague bacillus was abandoned.

In two cases the bacillus was isolated directly from the tissues of a naturally infected ground squirrel. Indeed the attempt to recover the plague bacillus in this manner has been made on only about a half-dozen occasions. The reason that the effort was not made to isolate the bacillus directly from the naturally infected animals more frequently, was because of the fact that by the time we received the squirrels at the laboratory in San Francisco they were at least 24 hours old; sometimes 48 or 72 hours had elapsed after the animal had been shot. As a result of this delay the tissues were usually badly contaminated with saprophytic organisms.

It was our intention in the early part of the work to verify by bacteriological methods at least one squirrel from each ranch on

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which infected animals were found, and this program has been faithfully carried out in nearly every case. However, as the number of infected animals received increased, in a few instances it was found impracticable to do this on account of lack of sufficient cages and of space in which to keep inoculated rats and guinea-pigs.

Mode of Handling and Examining.—A few of the squirrels were found dead by the men engaged in the anti-squirrel operations in Contra Costa and adjacent counties, a few were caught in traps, but the great majority were shot. These squirrels were packed in heavy sheet tin cans, each squirrel having been tagged to show the date on which it was taken and the person by whom it was shot, trapped, or found. The cans containing the squirrels were shipped to the Federal Plague Laboratory at San Francisco, a little chloroform having been placed in each can so that any ectoparasites might be killed. When the cans reached the laboratory a one per cent solution of trikresol or some similar disinfectant was used in sufficient quantity to cover the squirrels, after which they were nailed to shingles, the address recorded, and the animals dissected.

During the first few weeks of the work the medical officer personally examined every squirrel that was dissected. Later, as the number increased, it was found impracticable to do this and only those squirrels were examined that were laid aside by the laboratory assistants as suspicious. It is believed that no very material error resulted from this procedure, as during the whole of the time that all of the squirrels were scrutinized by the physician but two infected ones were found that had passed the men who dissected them. As soon as a suspicious lesion was found by an attendant, the dissection was suspended and the animal was laid aside for further and more detailed examination.

The location of lymphatic glands of ground squirrels is a point worth considering. The cervical, axillary, and pelvic glands are easily distinguished, being found in the usual situation of these glands in other rodents (rats and guinea-pigs). In the case of the inguinal glands, however, the location is somewhat different. In the squirrel there is a lymph node lying immediately above the pubis on each side and very near the middle line. In the ordinary dissection, this gland is very apt to be overlooked and as it is one which is very frequently involved in plague, it is important that it be exposed. We first learned of the location of this structure in doing certain work in the artificial infection of squirrels with plague. In addition to this gland, which we have for the sake of convenience called "the median inguinal gland," there is a chain of glands lying rather far back in the groin, sometimes extending nearly to the vertebral column. These structures we have called "the posterior inguinal glands." The great majority of squirrels do not have glands in the same location in the inguinal region as do rats and guinea-pigs; that is to say, about the middle of the groin.

THE GROSS LESIONS OF NATURAL SQUIRREL PLAGUE.

The lesions of four plague-infected squirrels were described by Wherry,[1] who also reviews the literature on squirrel plague.

Buboes.—The external appearances of buboes: It is not an unusual occurrence to be able to make a probable diagnosis of squirrel plague before an incision is made into the body. A swelling in the region of one of the superficial glands often gives a clue to the condition that will be found.

The buboes found in squirrels may be divided into two classes, first, those in which the gland is distinctly caseous; and second, those in which the gland is more or less purulent. In our experience thus far the latter has been much more frequently met than the former.

In the first class of cases, that is, in those with caseous buboes, the gland is rather firm and is usually yellowish red in color. There is frequently a small amount of hemorrhage into the surrounding tissue, and the contents may readily be shelled out when the gland is split across. These glands are usually about 1 c.c. in length by 0.5 c.c. in width. Smears made from buboes of this nature almost invariably show a large number of bacilli which agree in size, shape, and staining reactions with the plague bacillus. Very frequently numerous "coccoid" forms are present. The observation may be merely a coincidence but this class of buboes was found rather more frequently in the early part of our work than in the latter part; in fact now such buboes are rather rare, while during the first two weeks in which plague infected squirrels were found they were fairly common.

The second class of buboes (purulent) are quite different from the preceding. The lymph gland may be enlarged and succulent throughout, with the exception of one or more purulent foci no larger than a mustard seed. In other cases the purulent foci are very much larger, sometimes as large as a pea, and the gland may be entirely honeycombed with such small abscesses. In still other cases, the gland or chain of glands will be converted into a large pus sack, sometimes considerably more than 1 c.c. in diameter. In these purulent buboes pest-like bacilli either are very scarce, or as is true in the majority of cases, none at all are to be found by microscopical examination.

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[1] *Jour. Infect. Dis.*, 1908, 5, pp. 485–533.

In two or three cases, we have seen in smears from the bubo as well as in smears from the caseous lesions in the internal organs, objects which very strongly resemble the "involution" forms of plague bacilli when the organism is grown on salt agar. On one occasion we have seen these forms from a lung nodule of a case of human plague which died on the 16th day of the disease. This case was infected from squirrels.

In a considerable number of cases, one is in doubt as to whether a given bubo should be classed as caseous or as purulent. The content is a yellow, very tenacious, or flaky mass, which probably represents a transition stage between the purely caseous and the frankly purulent gland.

Spleen Lesions.—Enlargement of the spleen alone we have learned to regard as of but little significance because the size of the organ in the ground squirrel varies very markedly in the apparently normal animals. Indeed the largest spleens we have seen were in animals which were not suspected of being plague infected and which by inoculation experiments have proven to be free from the disease. However, in the plague infected animals the organ is frequently enlarged. It is usually deep red in color in contrast to the lilac tinge seen in the normal squirrel. Occasionally it is slate colored. The most common lesions of plague are caseous or purulent foci varying in size from a mustard seed to a mass as large as the last joint of one's index finger, and in number from one to a hundred or even more. In a few cases the caseous nodules seem to be in the process of organization.

Less frequently hemorrhagic areas are to be found which vary in size as do the caseous foci.

Liver Lesions.—In the liver are found purulent and caseous foci similar in size and number to those in the spleen. In a few cases the liver tissue has been very largely replaced by purulent masses, and in some of these cases it has been noted that there were large purulent masses scattered through the omentum and the mesentery. The organ is often adherent to the diaphragm.

Lung Lesions.—The lung lesions may be either caseous or purulent, and vary in size from a pin's head to a pea. Occasionally much larger nodules are found. The lesions are frequently surrounded by a deep red zone of congestion, and upon section are either caseous or purulent. Pleural adhesions over the site of the nodules are common.

Kidney Lesions.—Pea-sized abscesses have been found in a few cases.

General Appearance of the Squirrel.—Those animals in which lesions are very extensive are often markedly emaciated; sometimes in these cases the tissues are found to be jaundiced. On the other hand, in most of the cases in which the macroscopical evidence of plague is limited to a purulent gland, the animals are quite fat.

REMARKS ON THE GENERAL NATURE OF THE LESIONS.

The lesions of plague in most animals are generally uniform and well defined. The variation between individual cases in man, in the guinea-pig, and in the rat is slight and unimportant. These animals usually die between the third and the sixth or seventh day of the disease.

In the squirrel, however, there is a most marked diversity in the lesions found in different cases. They range all the way from a very small innocent-looking purulent lymphatic gland, up to extensive necrotic processes involving several of the chains of glands and almost all of the internal organs. Two examples illustrating the extremes of lesions found are given here.

The first case is one in which the lesions were very slight.

Squirrel No. 68.—Half grown female; shot on July 17, 1909, by C. The lesions are recorded as follows:

Lymphatic glands: Right median inguinal gland enlarged and shows several very small yellowish purulent points. The other glands are normal. Liver, spleen, and lungs negative.

Smears: Entirely negative for *B. pestis.* A guinea-pig (No. 68A) inoculated from the bubo material died on the third day with entirely characteristic lesions of plague and a pure culture of *B. pestis* was isolated from its tissues.

The next is an example of a squirrel showing very marked and widespread pathological lesions.

Squirrel No. 7.—Grown female, found dead by W. F. R. on June 18, 1909.

Lymphatic glands: Right cervical and bilateral median inguinal buboes each a little smaller than a pea and each full of yellow, creamy pus.

Liver: Numerous abscesses up to 2 mm. in diameter.

Spleen: Several distinct abscesses, largest about 1 cm. in diameter, numerous minute purulent points.

Lungs: About a dozen caseous foci, each one approximately 1 mm. in diameter.

Smears from the inguinal bubo show a few suspicious-looking organisms; from the liver show very typical pest-like bacilli.

On the surface of the spleen and of the liver there is found a considerable quantity of yellowish fibrinous exudate.

A guinea-pig inoculated subcutaneously from the liver of this squirrel died on the fourth day with characteristic lesions of plague; and a white rat inoculated by the cutaneous method also died on the fourth day, with the usual lesions of plague in the rat.

The question of course arises as to whether, especially in the cases where the anatomical changes are very extensive and widespread, some other organism plays a part in the production of the lesions. We can only say that these lesions have been invariably associated with pest bacilli. When the tissues reached us they were in such condition as to preclude a satisfactory bacteriological examination for other pathogenic organisms.

FREQUENCY OF VARIOUS LESIONS.

The gross lesions in these 70 cases of squirrel plague may be conveniently tabulated as follows:

Total showing buboes		58	(83%)
Buboes, single in 44 Buboes, multiple in 14	Cervical	17	
	Median, inguinal	14	
	Axillary	8	
	Pelvic	5	
Without buboes		12	(17%)
Total		70	

In the 44 cases with single buboes lesions of the internal organs were present as follows:

Spleen	10 times
Liver	6 "
Lungs	5 "
Spleen, liver, and lungs	3 "

Thirty-two (32) of the 44 cases had no lesions whatever beyond the bubo.

Multiple buboes (14) as follows:

Bilateral inguinal buboes, 7	Bilateral median inguinal only	2
	Bilateral median inguinal and bilateral pelvic	2
	Bilateral median inguinal and right cervical	1
	Bilateral median inguinal and right post inguinal	1
	Bilateral median inguinal right post inguinal and right axillary	1
Bilateral submaxillary, 1		

Median inguinal buboes and others, 4	Median inguinal and pelvic	3
	Median inguinal and post inguinal	1

Median inguinal and left axillary, 1

Median inguinal and right post inguinal and right submaxillary, 1

In these 14 cases, lesions of the internal organs were present as follows:

Spleen	3
Liver	5
Lungs	5
Spleen, liver, and lungs	2

Of these cases eight had no lesions whatever beyond the buboes. It will be observed that a much larger proportion of the cases with multiple buboes had lesions of the internal organs than of those with single buboes.

Cases without buboes (12) had lesions as follows:

Spleen	12
Liver	9
Lungs	2
Spleen, liver, and lungs	1

The anatomical findings would seem to justify the following observations:

It is probably fair to assume that all cases having lesions of the internal organs have been at some time septicemic.

In the cases where the only lesion is a bubo, it is very unlikely that the bacilli have ever invaded the blood stream.

Microscopical Examination of Smears.—Smears from one or more tissues were made in each case. The smears were stained with methylene blue or thionin, and a search made for pest-like bacilli. The results of the examinations are conveniently tabulated as follows:

Positive	17
Suspicious and doubtful	4
Negative	47
Not recorded	2
Total	70

On several occasions the smear from a bubo was negative while the organism was found in the splenic or hepatic nodules.

A word of explanation is necessary in regard to the recording of the results of the examination of stained smear preparations. In my experience with plague in man and in rodents, it is not advisable to spend much time looking for a few isolated organisms having the morphology of the pest bacillus, nor is any great stress laid upon the presence of a very few even quite characteristic organisms. Judging by these standards, in the great majority of cases the smears were regarded and recorded as negative. In a smaller percentage of the cases the examination of smears revealed such a large number of very typical pest-like bacilli as to make it almost certain that they were actually *B. pestis*. None of the cases included in this report depended on the examination of smears alone to support the diagnosis made from the gross anatomical appearances of the squirrel.

Results of animal inoculations.—As I have said in the introductory paragraph, the diagnosis in 68 of the 70 cases has been made (or confirmed) by animal inoculation. It is not my purpose to discuss here the lesions of plague in laboratory animals, but I will state that the lesions produced were those characteristic of plague in rats and in guinea-pigs.

In a considerable number of cases the inoculated animals died of subacute plague due probably to low virulence (or small number) of the organisms present in the squirrel tissues. In a great many cases, however, as will be seen from the tables below, the laboratory animals died of acute plague in the usual time, three, four, or five days.

CULTURAL CHARACTERISTICS OF THE ORGANISM ISOLATED.

The routine method of dealing with cultures was as follows:

> Cultures were made upon agar slants directly from the tissues of the squirrel or infected laboratory animal. These were examined at the end of 48 hours. If the growth was profuse or if the organism was evidently a chromogen, the culture was discarded without any further investigation. If the growth was found to be translucent, shiny, and if it adhered to the needle when touched, it was regarded as of sufficient importance to make subcultures on other media. Subcultures were always made on broth, and on salt agar; at the end of 48 hours a flocculent precipitate was observed in the broth culture and after a few days a delicate surface film which settled when the tube was agitated. Subcultures are also made on 3 per cent salt agar. A smear from this culture was examined microscopically after from 18 to 24 hours, when if the culture be one of *B. pestis* characteristic involution forms will be found. In the majority of cases subcultures were also made in litmus milk, which was usually rendered a trifle less alkaline at the end of 48 hours. The litmus milk cultures were also kept for at least 10 days for the purpose excluding *B. pseudo-tuberculosis rodentium* (Pfeiffer) which renders the milk very alkaline. In many cases we have also planted the organism in Dunham's solution, but have never observed the formation of indol. The plague bacillus, as is well known, does not produce indol. A few cultures have also been made on gelatin for the purpose of ascertaining the reaction to this medium.

Such a simple technic as that described is not to be recommended in any special case in which it is vitally important to isolate the bacillus from the tissue. In such cases it is better to use the plate method, as it is obvious that a small contamination may cause the loss of a culture in the method I describe. This was not a matter of special concern in the present work, as the bacillus had been isolated in a sufficient number of cases to leave no doubt as to the specific nature of the organism we were dealing with. The growth of plague bacillus on agar is so characteristic that a judgment based upon the appearance and nature of the growth on that medium very exceptionally proves to be erroneous.

We have used Yersin's anti-pest-serum for the purpose of verifying the diagnosis in this series of cases in but one instance. In this case, which was that of the first squirrel found infected during the present year (1909), two guinea-pigs were vaccinated from the bubo of the squirrel. One guinea-pig which weighed 290 gm. had been injected previously intraperitoneally with 3 c.c. of an old specimen of anti-plague serum. This guinea-pig died on the eleventh day. The internal organs were free from any lesions, but the superficial glands were all enormously enlarged and full of caseous material. A pure culture of the plague bacillus was isolated from one of these glands. In other words, this guinea-pig died of chronic or subacute plague. The other guinea-pig (the control), which weighed 335 gm., died on the fifth day with the typical lesions of acute plague and a pure culture of the bacillus was isolated from the liver. This one example is not insisted upon as being convincing in showing the protective power of anti-plague serum against the organism found in this particular squirrel, but it is very suggestive.

In the first case of plague found among the ground squirrels in California (1908) the subject of the relation of the infecting organism to anti-pest serum was worked out rather more carefully with results that I think would convince anyone that the serum exercised a high protective power. The results have been reported in the *Public Health Reports.*[1]

COMPARATIVE VIRULENCE FOR GUINEA-PIGS AND WHITE RATS.

In some of the early cases it was found that a white rat inoculated from a squirrel survived, while a guinea-pig inoculated from the same tissue succumbed to plague. With a view of determining exactly the comparative virulence for white rats and guinea-pigs, two series of rodents were inoculated. The results are shown in the following table:

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[1] Vol. 23, No. 37, September 11, 1909, p. 1292.

TABLE 1.

No. of Squirrel	Guinea-Pig Day of Death	White Rat—Day of Death
Inoculated Cutaneously		
1 (bubo)†	5 (c.)*	3 (c.)
65 (bubo)	8	Killed on 12th day; subacute plague
66 (bubo)	6 (c.)	Killed on 12th day; subacute plague
67 (liver nodule)	11 (c.)	Killed on 12th day; no lesions
68 (bubo)	4	3 (c.)
73 (bubo)	4 (c.)	4 (c.)
Inoculated Subcutaneously		
1 (bubo)	4 (c.)	2
69 (bubo)	5 (c.)	4
70 (bubo)	8	Killed on 11th day; no lesions
71 (bubo)	4 (c.)	5
72 (bubo)	5	3 (c.)

* (c.) indicates that a culture of *B. pestis* was isolated from the inoculated animal.

† The word in parenthesis after the number of the squirrel, shows the squirrel tissues used for the inoculations. These explanations also apply to the table on p. 686.

In each case the same piece of tissue was used to vaccinate or inoculate a guinea-pig, and to vaccinate or inoculate the corresponding white rat. It will be seen in both the subcutaneous and the cutaneous series that the infection was decidedly more virulent for guinea-pigs than for white rats as all of the guinea-pigs died of plague, while two of the white rats had no lesions whatever, and two developed subacute plague.

VIRULENCE FOR GUINEA-PIGS BY DIFFERENT MODES OF INOCULATION.

In order to determine the value of the cutaneous (vaccination) method of inoculation in squirrel plague two series of guinea-pigs were inoculated.

The technic was as follows: A piece of the suspected tissue was vigorously rubbed into the shaven belly of a guinea-pig. The same piece of tissue was then thrust into a pocket beneath the skin of another guinea-pig.

The table shows that of the 12 cases, two would have been missed by depending solely upon the cutaneous method of inoculation. Guinea-pigs of approximately the same weight were used in each case.

The question has been raised as to whether the plague encountered among these ground squirrels is identical in every respect with the plague found in rats and human beings. In answer to this question we can only say that either directly or by the use of laboratory animals we have isolated from these squirrels an organism which answers all of the cultural and pathogenic requirements for *B. pestis*.

The only difference observed between the strains of plague isolated from these squirrels and that from rats and man is, that the squirrel strain appears to be somewhat less virulent than the others. This reduction of virulence is shown by the fact that a considerable series of inoculated guinea-pigs lived on an average somewhat longer than guinea-pigs inoculated from natural plague rats or from cases of human plague; and second, the fact that a considerable percentage of the white rats are immune wholly or in part to this strain of the plague bacillus. In my experience I have not encountered among white rats a case of immunity to rat plague or to human plague, though such cases are said to occur. Cross immunization experiments are now in progress to determine the interrelationship of human plague, rat plague, and squirrel plague.

TABLE 2.

VIRULENCE FOR GUINEA-PIGS BY CUTANEOUS AND SUBCUTANEOUS INOCULATION.

No. of Squirrel	Guinea-Pig Inoculated Cutaneously Day of Death	Guinea-Pig Inoculated Subcutaneously Day of Death
1 (bubo)	5	4
6 (bubo)	5 (c.)*	5
43 (bubo)	4 (c.)	3
59 (bubo)	7	6
77 (bubo)	6	4
111 (bubo)	5 (c.)	6
120 (spleen)	Alive and well 12th day	6
123 (bubo)	6	6 (c.)
127 (bubo)	6	4 (c.)
135 (bubo)	Alive and well 16th day	5
137 (spleen)	5 (c.)	5
140 (spleen)	5 (c.)	4

SUMMARY AND CONCLUSIONS.

Plague in the ground squirrel is a disease that is readily recognized by the gross anatomical changes it produces.

The commonest lesion, and often the only one, is a bubo.

Many of the cases are probably examples of subacute or chronic plague.

In many cases the bacilli found in squirrel plague are highly virulent for guinea-pigs and white rats, in other cases the virulence is somewhat reduced.

Smear preparations are negative for pest-like bacilli in the majority of cases.

It is unsafe to trust to the cutaneous method of inoculation alone, as it will sometimes fail when the subcutaneous method yields positive results.